Shared hippocampal abnormalities in sporadic temporal lobe epilepsy patients and their siblings

Long, Lili ; Galovic, Marian ; Chen, Yayu ; Postma, Tjardo ; Vos, Sjoerd B ; Xiao, Fenglai ; Wu, Wenyue ; Song, Yanmin ; Huang, Sha ; Koepp, Matthias ; Xiao, Bo

Abstract: OBJECTIVE To examine the shared familial contribution to hippocampal and extrahippocampal morphological abnormalities in patients with sporadic temporal lobe epilepsy (TLE) and their unaffected siblings. METHODS We collected clinical, electrophysiological, and T1-weighted magnetic resonance imaging (MRI) data of 18 sporadic patients with TLE without lesions other than hippocampal sclerosis (12 right, 6 left), their 18 unaffected full siblings, and 18 matched healthy volunteers. We compared between-group differences in cortical thickness and volumes of five subcortical areas (hippocampus, amygdala, thalamus, putamen, and pallidum). We determined the subregional extent of hippocampal abnormalities using surface shape analysis. All our imaging results were corrected for multiple comparisons using random field theory. RESULTS We detected smaller hippocampal volumes in patients (right TLE: median right hippocampus 1.92 mL, interquartile range [IQR] 1.39-2.62, $P < .001$; left TLE: left hippocampus 2.05 mL, IQR 1.99-2.33, $P = .01$) and their unaffected siblings (right hippocampus 2.65 mL, IQR 2.32-2.80, $P < .001$; left hippocampus 2.39 mL, IQR 2.18-2.53, $P < .001$) compared to healthy controls (right hippocampus 2.94 mL, IQR 2.77-3.24; left hippocampus 2.71 mL, IQR 2.37-2.89). Surface shape analysis showed that patients with TLE had bilateral subregional atrophy in both hippocampi (right > left). Similar but less-pronounced subregional atrophy was detected in the right hippocampus of unaffected siblings. Patients with TLE had reduced cortical thickness in bilateral premotor/prefrontal cortices and the right precentral gyrus. Siblings did not show abnormalities in cortical or subcortical areas other than the hippocampus. SIGNIFICANCE Our results demonstrate a shared vulnerability of the hippocampus in both patients with TLE and their unaffected siblings, pointing to a contribution of familial factors to hippocampal atrophy. This neuroimaging trait could represent an endophenotype of TLE, which might precede the onset of epilepsy in some individuals.

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Shared hippocampal abnormalities in sporadic temporal lobe epilepsy patients and their siblings

Lili Long MD PhD,[1,2,] Marian Galovic MD,[2,3,4,*] Yayu Chen MD,[1] Tjardo Postma MD,[2] Sjoerd B Vos PhD,[2,3,5] Fenglai Xiao MD,[2,3] Wenyue Wu MD,[1] Yanmin Song MD PhD,[6] Sha Huang MD,[1] Matthias Koepp MD PhD, [2,3,#] Bo Xiao MD PhD [1,#]*

1. Department of Neurology, Xiangya Hospital, Central South University, China
2. Department of Clinical and Experimental Epilepsy, UCL Queen Square Institute of Neurology, London, United Kingdom
3. MRI Unit, Chalfont Centre for Epilepsy, Chalfont St Peter, United Kingdom
4. Department of Neurology, University Hospital Zurich, Switzerland
5. Centre for Medical Image Computing, University College London, London, United Kingdom
6. Department of Emergency, Xiangya Hospital, Central South University, China

[*] Lili Long and Marian Galovic contributed equally to this work

Corresponding Authors:
Bo Xiao, MD, PhD,
Xiangya Hospital, Central South University,
87 Xiangya Road,
Changsha, Hunan 410000, China.
E-mail: xiaobo1962_xy@163.com
Phone +86-731-84327216
Fax +86-731-84327401

Matthias Koepp, MD, PhD,
Institute of Neurology, University College London,
Box 29, Queen Square,
London, WC1N 3BG, UK.
E-mail: m.koepp@ucl.ac.uk
Phone +44 (0) 20 3448 8612
Fax +44 (0) 20 3448 8615

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ORCID number: Lili Long, 0000-0001-5078-8770; Marian Galovic, ; Matthias Koepp, ; Bo Xiao, 0000-0002-9800-1612

Summary

Objective: To examine the shared familial contribution to hippocampal and extrahippocampal morphological abnormalities in sporadic temporal lobe epilepsy (TLE) patients and their asymptomatic siblings.

Methods: We collected clinical, electrophysiological, and high-resolution structural neuroimaging data of 18 sporadic TLE patients without lesions other than hippocampal sclerosis (12 right, 6 left), their 18 unaffected full siblings, and 18 matched healthy volunteers. We compared between-group differences in cortical thickness and volumes of five subcortical areas (hippocampus, amygdala, thalamus, putamen, pallidum). We determined the subregional extent of hippocampal abnormalities using surface shape analysis. All our imaging results were corrected for multiple comparisons using random field theory.

Results: We detected smaller hippocampal volumes in patients (right TLE: median right hippocampus 1.92ml, interquartile range [IQR] 1.39-2.62, $p<0.001$; left TLE: left hippocampus 2.05ml, IQR 1.99-2.33, $p=0.01$) and their unaffected siblings (right hippocampus 2.65ml, IQR 2.32-2.80, $p<0.001$; left hippocampus 2.39 ml, IQR 2.18-2.53, $p<0.001$) compared to healthy controls (right hippocampus 2.94ml, IQR 2.77-3.24; left hippocampus 2.71ml, IQR 2.37-2.89). Surface shape analysis showed that TLE patients had bilateral subregional atrophy in both hippocampi (right > left). Similar but less pronounced subregional atrophy was detected in the right hippocampus of unaffected siblings. TLE patients had reduced cortical thickness in bilateral premotor/prefrontal cortices and the right precentral gyrus. Siblings did not show abnormalities in cortical or subcortical areas other than the hippocampus.

Significance: Our results demonstrate a shared vulnerability of the hippocampus in both TLE patients and their unaffected siblings, pointing to a contribution of familial factors to hippocampal atrophy. This neuroimaging trait could represent an endophenotype of TLE, which might precede the onset of epilepsy in some individuals.

Key words: temporal lobe epilepsy; hippocampus; surface shape analysis; cortical thickness; endophenotype

Abbreviations: CAT = Computational Anatomy Toolbox; IQR = interquartile range; MMSE = Mini Mental State Examination; TLE = temporal lobe epilepsy; TIV = total intracranial volume.

Key points

We detected hippocampal abnormalities in unaffected siblings of people with TLE using two methods (volumetry and surface shape analysis), pointing to a contribution of familial factors to hippocampal atrophy.

Shared familial contribution to hippocampal atrophy could reflect an imaging endophenotype of TLE, which might precede the onset of epilepsy in some individuals.

Cortical thinning observed in our TLE patients could be driven by the environmental factors rather than genetic factors.

Introduction

Hippocampal sclerosis is the hallmark of temporal lobe epilepsy (TLE), the most common focal epilepsy syndrome in adults. [1] Whether hippocampal atrophy is the cause or consequence of seizures remains controversial. Previous studies widely considered that hippocampal sclerosis might be an acquired phenomenon secondary to postnatal injury such as prolonged febrile seizures. [2,3] Patients with prolonged febrile seizures in early childhood have smaller, more asymmetric, or malrotated hippocampi. [4-6] Moreover, the evolution of hippocampal changes has been documented on magnetic resonance imaging (MRI) after an emergency event.[7,8] Several large prospective studies of children with febrile seizures failed to show a convincing association between a precipitating injury and hippocampal sclerosis. [9-12]

The genetic contribution to sporadic TLE is another topic of ongoing debate. Relatives of people with focal epilepsy have a 2.6-times higher risk for epilepsy compared to the general population. [13] Two large multicentre studies, however, failed to define common genetic variants associated with the risk of focal epilepsy. [14,15] Using more detailed phenotyping, a subsequent study described a common risk variant of temporal lobe epilepsy with hippocampal sclerosis and febrile seizures, [16] demonstrating that well-defined disease phenotypes combined with specific neuroimaging traits lend themselves to the study of genetic variation. In this regard, the concept of imaging endophenotypes might be helpful to define potentially heritable neuroimaging traits. An endophenotype (or intermediate phenotype) is a quantitative biological trait that is reliable and reasonably heritable, i.e. shows greater prevalence in unaffected relatives of patients than in the general population. [17] Hippocampal atrophy, due to its high prevalence and relative specificity to epilepsy arising from the temporal lobes, [18] represents an excellent candidate for such an imaging trait.

Studies including subjects with familial TLE indicated that structural abnormalities within the hippocampus may be determined by a strong genetic predisposition and thus represent a

risk factor for TLE. [19,20] Alhusaini and colleagues [21] reported alterations of cortical morphology in anteromedial regions of the ipsilateral temporal lobe in unaffected siblings of patients with sporadic TLE, and these findings were recently replicated by another group of investigators.[22] There is, however, little evidence for heritability of hippocampal traits in sporadic TLE. Previous studies used an automated segmentation pipeline that might be insensitive to small or sclerotic hippocampi, [23,24] and thus did not find significant volumetric changes in siblings of sporadic TLE patients, although some observed a trend for bilaterally reduced hippocampal volumes. [21,25,26]

Here, we analysed the shared familial contribution to cortical and subcortical brain morphology in TLE patients and their unaffected siblings with the aim to determine a neuroimaging endophenotype of TLE. We specifically focused on the hippocampus, the hallmark of structural changes in TLE, and were interested whether hippocampal abnormalities can be observed in asymptomatic siblings in the absence of precipitating injuries, seizures, or medication intake.

Methods

Subjects

We screened consecutive patients with temporal lobe epilepsy (TLE) under follow-up at the Outpatient Clinical Neurology Department of Xiangya Hospital, Central South University (Hunan Province, China) from March 2015 to March 2018. We included patients with a diagnosis of sporadic TLE who had an asymptomatic full sibling. Recruitment was complicated by the one-child policy in China, which is less strictly adhered to in rural China. TLE was diagnosed by certified neurologists specializing in epilepsy based on clinical history, seizure semiology, long-term video-electroencephalography (EEG), and magnetic resonance imaging (MRI). We excluded subjects with brain lesions other than hippocampal sclerosis and people

with concomitant hereditary, psychiatric, or neurologic conditions other than epilepsy.

We matched the patients' asymptomatic full siblings and unrelated healthy volunteers for age and sex. Siblings and healthy controls did not have a history of epileptic seizures, neuropsychiatric, or genetic disorders and they presented with a normal neurological examination and structural MRI. Excluded were those with a history of illicit drug abuse, febrile seizures, or other precipitating injuries. In healthy volunteers, there was no history of seizures or epilepsy in up to three generations of relatives. One sibling showed occasional asymmetrical generalised atypical sharp wave activity with frontal and temporal maximum on EEG but had no history of detectable seizures or epilepsy on detailed questioning. All other siblings and healthy volunteers had an unremarkable routine EEG.

The Ethics Committee of Xiangya Hospital of Central South University approved this study, and all participants gave written informed consent.

MRI data acquisition and processing

High-resolution three-dimensional (3D) brain anatomical images were acquired on a 3.0T GE Signal HDx scanner, by using a T1-weighted MP-RAGE sequence according to the following parameters: TE=2.98 ms; TR=7792 ms; TI=800 ms; field of view=256×256 mm; number of slices=188; slice thickness=1.0 mm; flip angle=7 degrees; voxel size= 1×1×1 mm3.

Cortical thickness estimation

We estimated cortical thickness using a projection-based thickness method implemented in the Computational Anatomy Toolbox (CAT12, *www.neuro.uni-jena.de/cat/)* in Statistical Parametric Mapping (SPM12, *www.fil.ion.ucl.ac.uk/spm/software/spm12/*). This approach was validated using spherical and brain phantoms confirming accurate measurements under a wide set of parameters for several thickness levels. [27] CAT12 showed excellent test-retest reliability

($R^2 = 0.986$) and was validated against other cortical surface reconstruction methods, showing fewer thickness measurement errors than comparable approaches. [27-29] After estimation of cortical thickness and the central surface, we carried out topology correction, spherical mapping, and spherical registration. Data were inspected visually and using the retrospective quality assurance protocol implemented in CAT12.

Subcortical segmentation and volumetry

We used Hipposeg (*https://hipposeg.cs.ucl.ac.uk*) to automatically extract the initial hippocampal segmentations [30]. Hipposeg delineates the hippocampus with no more variability than seen between expert human raters and is robust to atrophic hippocampi. Next, one blinded rater (LL) received anonymized hippocampal masks and corrected segmentation errors according to a well-established protocol. [31]

To assess intra-rater variability of this combined manual-automated approach, one blinded rater manually corrected Hipposeg segmentation in randomly selected 10 TLE patients on two different occasions 3 months apart and compared the resulting masks using Dice coefficients.[32] To determine inter-rater variability, a second blinded rater corrected Hipposeg segmentations of 10 randomly selected TLE patients using the same segmentation protocol. A high intra-rater (0.98 ± 0.01) and inter-rater (0.96 ± 0.02) reliability demonstrated a high consistency of the combined manual-automated method, exceeding the reliability reported for an entirely manual method (intra-rater 0.89 ± 0.02, inter-rater 0.83 ± 0.02). [30]

Volumes of other subcortical structures relevant in epilepsy (thalamus, amygdala, putamen, pallidum) [33] and the total intracranial volume (TIV) were extracted using a parcellation algorithm based on Geodesic Information Flows (GIF) [34] freely available within NiftyWeb (*http://cmictig.cs.ucl.ac.uk/niftyweb*, UCL Centre for Medical Image Computing, UK).

Additionally, an experienced neuroradiologist visually assessed all MRI scans. Hippocampal sclerosis was defined visually as reduced hippocampal volume overall and in comparison to the contralateral side (i.e. increased asymmetry), loss of internal architecture, or signal increase on T2-weighted imaging.

Hippocampal shape analysis

Final binary hippocampal segmentations were converted to 3D surface meshes and parametrised with a spherical harmonics point distribution model (SPHARM-PDM). [35] In short, to ensure spherical topology of hippocampal segmentations uneven boundaries were minimally smoothed while the original binary surface was used as a constraint ensuring marginal loss of ± 3 voxels of the original surface. Subsequently, these surfaces were represented by spherical harmonics (SPHARM), which were then sampled onto triangulated surfaces (SPHARM-PDM), producing detailed surface information across 1002 vertices. We generated a mean mesh template from 18 healthy volunteers and all hippocampal surfaces were aligned to this mean mesh. Hippocampal shapes were visually checked for both surface mesh and alignment failures. Displacement values were generated using a point to mesh approach calculating the normal distance between the mean template surface and each point on an individual's hippocampal surface mesh. An inward displacement (indicated by a negative displacement value) typically corresponds to atrophy, outward displacement (positive displacement value) to hypertrophy. All preprocessing was done separately for left and right hippocampi.

Statistical analysis

We first compared the overall group of TLE patients and their siblings to healthy volunteers. We performed subsequent subgroup-analyses in left- and right-lateralised patients. We also split siblings into left- and right-lateralised groups based on the epilepsy lateralisation

of their relatives with epilepsy. The rationale behind this approach was to determine whether genetic factors contribute to morphological abnormalities in unaffected siblings in a lateralised manner, similar to the TLE patients they are related to.

Demographic characteristics were compared with the Chi-Square test or independent-sample T-test. Volumetric group-differences (patients vs. healthy controls; siblings vs. healthy controls) were analysed using a full-factorial general linear model with age, sex, educational level, Mini Mental State Examination (MMSE) scores, and TIV as covariates of no interest. Asymmetry indices were calculated as the difference of left and right hippocampal volumes divided by their mean (negative values indicating more left-lateralised atrophy). Data are presented as N (%) or median (interquartile range [IQR]). We also report bias-corrected p-values (p_{BS}) and effect sizes (ß) with 95% confidence intervals (CI) that were calculated for the volumetric results using 1000 bootstrapped random samples. These values are optimism-corrected and generalizable, because they are less dependent on sample composition. Calculations were performed in SPSS statistical analysis package, version 25.0 (IBM-SPSS, Armonk, NY, USA).

We statistically compared vertex-wise cortical thickness and point-wise displacement values on hippocampal surfaces using fixed-effect linear models implemented in SurfStat (*http://www.math.mcgill.ca/keith/surfstat/*). We used age, sex, level of education, and MMSE scores as covariates of no interest. TIV was used as an additional covariate for hippocampal shape analysis.

We report all our findings thresholded at $p<0.05$ corrected for multiple comparisons using random field theory for nonisotropic images on a cluster level. [36]

Results

Demographic characteristics

In total, we included 18 TLE patients (10 female; age 29y, interquartile range IQR 20-41y), 18 unaffected full siblings (11 female; age 30y, IQR 24-42), and 18 healthy volunteers (10 female; age 28y, IQR 22-43y). Demographic characteristics are displayed in Table 1. Visual MRI assessment detected hippocampal sclerosis in seven TLE patients and in one unaffected sibling (Table 1). No healthy volunteers had hippocampal sclerosis on visual evaluation.

There were no between-group differences in age or sex. Patients had significantly lower MMSE scores compared to controls (p=0.006). There was a trend for lower MMSE scores in siblings (p=0.07) and lower level of education in patients (p=0.12) and siblings (p=0.31) compared to controls. All subsequent statistical analyses were adjusted for these variables.

Overall group findings

The overall group of all TLE patients (Figure 1A; n=18; 12 right, 6 left) had reduced cortical thickness in the right superior frontal and precentral gyri (932 vertices, 4.7 resels, p=0.0005) and bilateral middle frontal gyri (right, 722 vertices, resels > 2.2, p<0.04; left, 359 vertices, 2.3 resels, p=0.03) compared to healthy volunteers. TLE patients had reduced overall volume of both hippocampi, more on the right (TLE median 2.37 ml, IQR 1.79-2.67, vs. controls 2.94 ml, IQR 2.77-3.24, ~~ß = -0.65 [95% CI -0.96 to -0.36],~~ p<0.001~~, p_{BS}=0.003~~) than on the left (TLE 2.43 ml, IQR 2.07-2.67, vs. controls 2.71 ml, IQR 2.37-2.89, ~~ß = -0.30 [95% CI -0.48 to -0.06],~~ p=0.02~~, p_{BS}=0.02~~). Detailed hippocampal volume data are displayed in Table 2. Other subcortical areas (amygdala, thalamus, putamen, pallidum) showed no differences. Hippocampal surface morphology detected focal atrophy in the lateral rim (224 points, 8.7 resels, p<0.00001), inferior medial rim (75 points, 4.2 resels, p=0.0003) and superior medial head (36 points, 2.5 resels, p=0.006) of the right hippocampus and medial body of the left hippocampus (44 points, 2.1 resels, p=0.01).

Unaffected siblings (Figure 1B; n=18) did not show abnormal cortical thinning compared to healthy volunteers. Siblings had reduced overall volume of both left (siblings 2.39 ml, IQR 2.18-2.53, vs. controls 2.71 ml, IQR 2.37-2.89, ß = -0.37 [95% CI -0.53 to -0.20], p<0.001, p_{BS}=0.002) and right (siblings 2.65 ml, IQR 2.32-2.80, vs. controls 2.94 ml, IQR 2.77-3.24, ß = -0.43 [95% CI -0.61 to -0.21], p<0.001, p_{BS}=0.001) hippocampi. Hippocampal shape analysis demonstrated focal atrophy in the lateral tail (48 points, 1.9 resels, p=0.02) and head (37 points, 1.6 resels, p=0.04) of the right hippocampus. Other subcortical areas showed no differences in siblings.

Left TLE patients and their siblings

Left TLE patients (Figure 2A; n=6) showed cortical thinning in the bilateral superior frontal, middle frontal, and precentral gyri (left, 1029 vertices, 6.6 resels, p=0.00004; right, 1334 vertices, 6.7 resels, p=0.00003) and the left postcentral and superior parietal gyri (680 vertices, 4.7 resels, p=0.0005). They had a significantly smaller overall volume of the left hippocampus (left TLE 2.05 ml, IQR 1.93-2.33, vs. controls 2.71 ml, IQR 2.37-2.89, ß = -0.42 [95% CI -0.72 to -0.10], p=0.01, p_{BS}=0.03) and a trend for smaller volume of the right hippocampus (left TLE 2.68 ml, IQR 2.49-2.71, vs. controls 2.94 ml, IQR 2.77-3.24, ß = -0.39 [95% CI -0.67 to -0.10], p=0.06, p_{BS}=0.06). Focal atrophy was detected in the left inferior hippocampal head (39 points, 2.2 resels, p=0.01) and tail (30 points, 1.7 resels, p=0.03).

Unaffected siblings of left TLE patients (Figure 2B; n=6) did not show abnormal cortical thinning or focal abnormalities of the hippocampal surface. They had reduced overall volume of both left (siblings 2.44 ml, IQR 2.31-2.66, vs. controls 2.71 ml, IQR 2.37-2.89, ß = -0.34 [95% CI -0.44 to -0.19], p=0.007, p_{BS}=0.009) and right (siblings 2.71 ml, IQR 2.50-2.89, vs. controls 2.94 ml, IQR 2.77-3.24, ß = -0.41 [95% CI -0.58 to -0.14], p=0.02, p_{BS}=0.02) hippocampi.

Right TLE patients and their siblings

We did not detect significant cortical thinning in right TLE patients (Figure 3A; n=12). Subcortical volumetry showed reduced overall volume of the right (right TLE 1.92 ml, IQR 1.39-2.62, vs. controls 2.94 ml, IQR 2.77-3.24, ß = -0.75 [95% CI -1.1 to -0.36, p<0.001, p_{BS}=0.007) but not the left (right TLE 2.55 ml, IQR 2.29-2.91, vs. controls 2.71 ml, IQR 2.37-2.89,] ß = -0.16 [95% CI -0.36 to 0.08], p=0.17, p_{BS}=0.26) hippocampus. Focal atrophy was found in a large area affecting mainly the lateral side, inferior surface, and superomedial head of the right hippocampus (404 points, 18.5 resels, p<0.00001).

Unaffected siblings of right TLE patients (Figure 3B; n=12) did not show abnormal cortical thinning. They had reduced overall volume of both left (siblings 2.31 ml, IQR 2.09-2.51, vs. controls 2.71 ml, IQR 2.37-2.89, ß = -0.40 [95% CI -0.66 to -0.12], p=0.002, p_{BS}=0.009) and right (siblings 2.57 ml, IQR 2.28-2.78, vs. controls 2.94 ml, IQR 2.77-3.24, ß = -0.46 [95% CI -0.70 to -0.17], p=0.003, p_{BS}=0.009) hippocampi. Hippocampal shape analysis detected focal atrophy in the lateral body/tail (83 points, 3.4 resels, p=0.001) and inferior head (35 points, 1.6 resels, p=0.04) of the right hippocampus.

Other analyses

Hippocampal volumes of right TLE patients were asymmetric pointing to smaller volumes on the right (median asymmetry index 30%, IQR 0 to 68, p<0.001), whereas there was no asymmetry in their relatives (-1%, IQR -7 to 1, p=0.23) when compared to healthy volunteers (-4%, IQR -12 to -1). Similarly, volumes of left TLE patients were asymmetric with smaller volumes on the left (-16%, IQR -30 to 4, p=0.048), but no asymmetry was observed in their relatives (-8%, IQR -10 to 1, p=0.79).

We conducted a sensitivity analysis excluding one sibling who had an abnormal EEG.

Excluding this dataset did not alter the overall results (see online supplement, section 1). We also correlated clinical factors (duration of epilepsy, history of secondarily generalized seizures, seizure frequency, and number of antiepileptic drugs) with brain morphology in people with TLE and provide the detailed results in the online supplement, section 2. A subgroup analysis in patients with TLE and hippocampal sclerosis (n=7) is given in the online supplement, section 3. Lastly, we found abnormalities of cortical surface area in TLE patients but not in their siblings, as described in the online supplement, section 4.

Discussion

We performed detailed morphological analyses in a cohort of sporadic temporal lobe epilepsy patients, their asymptomatic full siblings, and matched healthy controls. We found shared hippocampal abnormalities that were mostly asymmetric in patients and bilateral in siblings, and were detected using two independent methods (volumetry and surface shape analysis). Structural changes in siblings were restricted to the hippocampus and were not found in other subcortical or cortical areas. These shared traits point to a familial vulnerability of the hippocampus in sporadic TLE patients and their siblings and could represent an imaging endophenotype of TLE.

Little is known about the genetic contribution to sporadic TLE and defining genetic risk factors has proven difficult. [14-16] Our findings stand out in this regard because they strongly suggest a familial contribution to hippocampal atrophy in sporadic TLE. We found hippocampal morphological abnormalities in siblings who never had seizures, did not take regular medication, and had no history of febrile seizures or neurologic disease. This is of interest because it shows that hippocampal abnormalities can develop in the absence of precipitating injuries and the effects of seizures or medication. Our results support the notion that, in some cases, disruption of hippocampal structural integrity might precede the onset of

seizures rather than being the consequence of epilepsy. They also indicate that genetic factors are likely to contribute to hippocampal atrophy observed in TLE and their siblings. Recently, common genetic variants associated with hippocampal volume were identified in the general population. [37,38] It will need to be determined whether these or other variants contribute to the imaging endophenotype of hippocampal atrophy in TLE and their siblings.

Another relevant observation is that siblings did not experience seizures despite presenting with a hippocampal imaging trait, which indicates that additional environmental and/or genetic factors are necessary to develop epilepsy. There are three plausible explanations. Firstly, mild hippocampal disturbances observed in siblings might not be severe enough to cause the emergence of seizures, whereas a more severe phenotype or genotype in TLE patients could lead to epilepsy. Secondly, a combination of the genetic predisposition to hippocampal structural impairment [39] and additional environmental factors, e.g. HHV-6 viral infection, febrile seizures or other precipitating injuries, could be the cause of more severe hippocampal damage and, subsequently, seizures in TLE. Lastly, hippocampal atrophy could be an epiphenomenon of a genetic predisposition to epilepsy without a causative role in disease development. This is less likely because hippocampal atrophy is not prevalent in the general nonepileptic population, [18] surgical removal of the atrophic hippocampus frequently alleviates or stops seizures, [40] and a wealth of animal research proposes a central role of the hippocampal formation in epileptogenesis. [41]

Alhusaini and colleagues summarized the literature on familial traits in epilepsy before 2016, and found a probable familial component to hippocampal structural alterations particularly in TLE patients with a strong family history for seizures. [42] Previous cohorts of sporadic TLE, including two recent studies,[22,43] did not find significant hippocampal changes in unaffected relatives of patients with sporadic TLE, [21,25,26] whereas one study observed a trend for bilaterally smaller hippocampi in asymptomatic siblings, [21] similar to our results. One

reason for the failure to detect hippocampal atrophy in the relatives of sporadic patients in previous studies is the use of an automated segmentation pipeline implemented in Freesurfer, that is known to be unreliable in small or sclerotic hippocampi. [23,24] A strength of our study is the implementation of a highly reliable (intra-rater reliability 0.98 ± 0.01, inter-rater reliability 0.96 ± 0.02) semi-automated segmentation approach based on Hipposeg, that has been developed specifically for epilepsy and remains robust when applied to sclerotic hippocampi. Hipposeg (Pearson correlation coefficient r = 0.93 - 0.94) performs better than Freesurfer (r = 30.69 - 0.79) when compared to manual segmentations.[23,24,30] The increased accuracy of hippocampal segmentations in our study assured a higher sensitivity to detect differences between siblings and healthy volunteers. In addition, we used hippocampal shape analysis, a novel sensitive approach to detect morphological abnormalities on a subregional level that allows to reveal focal atrophy that might be missed by volumetry alone.

One previous study included a mixed cohort of familial and sporadic TLE patients and described reduced hippocampal volumes in their unaffected relatives, particularly in those related to people with familial TLE.[20] There are important differences between this previous study and our cohort and we expand on the previous findings. Because the inclusion of familial TLE cases might overestimate the genetic contribution to neuroimaging traits, we have only included sporadic TLE cases. We also excluded siblings who had a history of precipitating injuries or febrile seizures, to minimise the effect of these events on hippocampal morphology. We did not restrict our calculations to the hippocampus but performed whole-brain cortical and subcortical analysis. Lastly, we evaluated the subregional extent of hippocampal abnormalities with surface-shape analysis.

We found bilaterally smaller hippocampi in the overall group of unaffected siblings and in both subgroups of siblings related to left or right TLE patients. In contrast, visual assessment found definitive hippocampal sclerosis in only one sibling. This confirms that hippocampal

changes in siblings are typically mild and usually do not fulfil the visual criteria for definitive hippocampal sclerosis. We did not observe hippocampal volumetric asymmetry in siblings, whereas focal hippocampal changes on surface shape analysis were more likely to be detected in the right hippocampus. This suggests that familial contribution to hippocampal damage is usually bilateral, potentially with a slight right-sided predominance. ~~Because abnormal surface shapes in the right hippocampus were only seen in relatives of right and not of left TLE patients, familial factors might contribute to hippocampal atrophy in both patients and siblings in a lateralised manner. This is in keeping with studies showing ipsi-lateral morphological changes in the temporal pole in siblings of patients with unilateral TLE.~~ [21,22] ~~Larger studies will be needed to confirm the laterality of these findings.~~

Focal hippocampal changes detected with surface shape analysis in TLE patients and their siblings showed largely similar patterns. In both groups, surface abnormalities were most pronounced in the right lateral hippocampus mainly corresponding to the CA1 subfield,[44] in accordance with histological findings related to hippocampal sclerosis. [45] This spatial overlap might suggest that familial factors affect the hippocampus in a subregionally specific manner.

In contrast to unaffected siblings, TLE patients had pronounced asymmetry of hippocampal volumes. This asymmetry might be the consequence of a precipitating injury predominantly affecting one hemisphere [5] or due to lateralised hippocampal damage by neuronal disconnection or seizures once unilateral epilepsy is established.

Patterns of cortical thinning detected in TLE patients were similar to those described in previous research, mainly affecting bilateral precentral and prefrontal areas. [33,46] In line with previous findings, left TLE patients were more likely to show widespread cortical thinning than people with right TLE. [33,47] In contrast, unaffected siblings did not show morphological abnormalities outside of the hippocampus. This observation might suggest that genetic factors shared between TLE patients and their siblings mainly affect the hippocampus and have little

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influence on other subcortical or cortical areas. Cortical thinning observed in TLE patients could be, thus, driven by the spread of epileptic discharges or ongoing seizures rather than genetic factors. In support of this, cortical thinning was previously associated with seizure frequency and duration of epilepsy[33,48] and we also found a correlation with duration of epilepsy (online supplement, section 2). On the other hand, two previous studies found altered cortical morphology driven by surface area contractions in siblings of TLE patients,[21,22] but there were no changes in cortical thickness.[43] In our study, significant surface area abnormalities were found in TLE patients but not in their siblings (online supplement, section 4). Further larger studies will be needed to address this issue.

Our study has limitations. Firstly, the included cohort was small. Nevertheless, we obtained detailed phenotypic information and used robust and accurate methods to analyse the data, allowing us to detect shared hippocampal abnormalities in patients and siblings using two different approaches (volumetry and shape analysis) despite the small number of subjects. We report bias-corrected effect-sizes and overoptimism-corrected p-values (p_{BS}) that increase the generalizability of our results because the corrected metrics are independent of sample composition. Nevertheless, larger studies with higher statistical power might extend these findings in future. Secondly, the included subjects were all ethnic Chinese and replication in other ethnic groups or mixed cohorts will be necessary. Lastly, familial factors shared by siblings and TLE patients include not only genetic but also shared environmental factors. The impact of shared environmental factors on our results cannot be completely eliminated, but their contribution to brain morphology is likely smaller than that of shared genetic factors.

Conclusion

We found shared morphological abnormalities in the hippocampus of both patients with TLE and their asymptomatic siblings. Hippocampal atrophy occurred in unaffected siblings in

the absence of precipitating injuries, seizures, or medication intake. This challenges the notion that hippocampal atrophy is an exclusively acquired phenomenon and rather lends support to the idea that, in some cases, hippocampal changes might precede the onset of epilepsy and could be the cause rather than the consequence of seizures. Shared familial contribution to hippocampal damage could reflect an imaging endophenotype of TLE, representing the underlying genetic and shared environmental risk of TLE in both disease-affected and -unaffected siblings. Such an imaging trait could be used as a potential biomarker for future genetic studies to identify common variants associated with risk of sporadic TLE. Early hippocampal morphological changes could also serve as prognostic markers for the development of epilepsy after an initial insult or a first seizure and this should be evaluated in future prospective trials.

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Disclosure of Conflicts of Interest

None of the authors has any conflict of interest to disclose.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Tables

	TLE patients (n = 18)	Unaffected siblings (n = 18)	Healthy volunteers (n = 18)
Sex			
Female	10 (56%)	11 (61%)	10 (56%)
Male	8 (44%)	7 (39%)	8 (44%)
Age and duration (*years*)			
Age at scan	29 (20-41)	30 (24-42)	28 (22-43)
Age at seizure onset	14 (11-26)		
Duration of epilepsy	11 (6-16)		
Level of Education (*years*)	9 (9-16)	12 (9-16)	16 (9-16)
MMSE	29 (25-30)	30 (29-30)	30 (30-30)
Handedness			
Right	18 (100%)	18 (100%)	18 (100%)
Left	0 (0%)	0 (0%)	0 (0%)
Seizures			
CPS	6 (33%)		
CPS+SGS	10 (56%)		
CPS +SPS+SGS	2 (11%)		
Status epilepticus	0 (0%)		
CPS frequency			
≤once per month	4 (22%)		
2~4 times per month	4 (22%)		
＞4 times per month	10 (56%)		
Epilepsy lateralisation			
Right	12 (67%)		
Left	6 (33%)		
Hippocampal sclerosis (visual)	7 (39%)	1 (6%)	0 (0%)
History of febrile convulsion	5 (28%)	0 (0%)	0 (0%)
Number of antiepileptic drugs			
None	2 (11%)	18 (100%)	18 (100%)
1	10 (56%)	0 (0%)	0 (0%)
2	5 (28%)	0 (0%)	0 (0%)
3	1 (56%)	0 (0%)	0 (0%)

Table 1: Demographic and clinical characteristics. Data presented as N (%) or median (interquartile range). MMSE, Mini Mental State Examination; CPS, complex partial seizures; SGS, secondary generalized seizures; SPS, simple partial seizures; AED, antiepileptic drugs.

	Median (ml)	IQR (ml)	Statistical ~~Statistics~~comparison ~~(~~vs. controls			
			ß	95% CI	p_{uncorr}	p_{BS}
~~Overall TLE groups~~All subjects						
~~(~~Right hippocampal volume~~)~~						
Patients	2.37	1.79-2.67	-0.65	-0.96 to -0.36	<0.001	0.003
Siblings	2.65	2.32-2.80	-0.43	-0.61 to -0.21	<0.001	0.001
Controls	2.94	2.77-3.24	--	--	--	--
~~Overall TLE groups~~						
~~(~~Left hippocampal volume~~)~~						
Patients	2.43	2.07-2.67	-0.30	-0.48 to -0.06	0.02	0.02
Siblings	2.39	2.18-2.53	-0.37	-0.53 to -0.20	<0.001	0.002
Controls	2.71	2.37-2.89	--	--	--	--
Left TLE ~~groups~~						
~~(~~Left hippocampal volume~~)~~						
Patients	2.05	1.93-2.33	-0.42	-0.72 to -0.10	0.01	0.03
Siblings	2.44	2.31-2.66	-0.34	-0.44 to -0.19	0.007	0.009
Controls	2.71	2.37-2.89	--	--	--	--
~~Left TLE groups~~						
~~(~~Right hippocampal volume~~)~~						
Patients	2.68	2.49-2.71	-0.39	-0.67 to -0.10	0.06	0.06
Siblings	2.71	2.50-2.89	-0.41	-0.58 to -0.14	0.02	0.02
Controls	2.94	2.77-3.24	--	--	--	--
Right TLE ~~groups~~						
~~(~~Right hippocampal volume~~)~~						
Patients	1.92	1.39-2.62	-0.75	-1.1 to -0.36	<0.001	0.007
Siblings	2.57	2.28-2.78	-0.46	-0.70 to -0.17	0.003	0.009
Controls	2.94	2.77-3.24	--	--	--	--
~~Right TLE groups~~						
~~(~~Left hippocampal volume~~)~~						
Patients	2.55	2.29-2.91	-0.16	-0.36 to 0.08	0.17	0.26
Siblings	2.31	2.09-2.51	-0.40	-0.66 to -0.12	0.002	0.009
Controls	2.71	2.37-2.89	--	--	--	--

Table 2: Statistical results of hippocampal volumes among three groups. Volumetric group-differences (patients vs. healthy controls; siblings vs. healthy controls) were analysed using a full-factorial general linear model with age, sex, educational level, Mini Mental State Examination (MMSE) scores, and total intracranial volume (TIV) as covariates of no interest. IQR, interquartile range; ß, effect sizes; CI, confidence intervals; p_{uncorr}, uncorrected p-values; p_{BS}, bias-corrected p-values.

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Figure legends

Figure 1: *Overall group findings.*

Displayed are comparisons of TLE patients (**Panel A**, n=18) and their unaffected siblings (**Panel B**, n=18) with unrelated healthy volunteers (n=18). The top panels show significant clusters of cortical thinning on medial and lateral views of the cerebral hemispheres. The middle panels show volumetric findings for the hippocampus (H), amygdala (A), thalamus (T), putamen (Pu), and pallidum (Pa) in the left and right hemispheres. The bottom panels show significant focal inward deformations (i.e. atrophy) on superior, lateral, and inferior views of the left and right hippocampal surface reconstructions.

Figure 2: *Left TLE patients and their siblings.*

Displayed are comparisons of left TLE patients (**Panel A**, n=6) and their unaffected siblings (**Panel B**, n=6) with unrelated healthy volunteers (n=18). The top panels show significant clusters of cortical thinning on medial and lateral views of the cerebral hemispheres. The middle panels show volumetric findings for the hippocampus (H), amygdala (A), thalamus (T), putamen (Pu), and pallidum (Pa) in the left and right hemispheres. The bottom panels show significant focal inward deformations (i.e. atrophy) on superior, lateral, and inferior views of the left and right hippocampal surface reconstructions.

Figure 3: *Right TLE patients and their siblings.*

Displayed are comparisons of right TLE patients (**Panel A**, n=12) and their unaffected siblings (**Panel B**, n=12) with unrelated healthy volunteers (n=18). The top panels show significant clusters of cortical thinning on medial and lateral views of the cerebral hemispheres. The middle panels show volumetric findings for the hippocampus (H), amygdala (A), thalamus (T),

putamen (Pu), and pallidum (Pa) in the left and right hemispheres. The bottom panels show significant focal inward deformations (i.e. atrophy) on superior, lateral, and inferior views of the left and right hippocampal surface reconstructions.

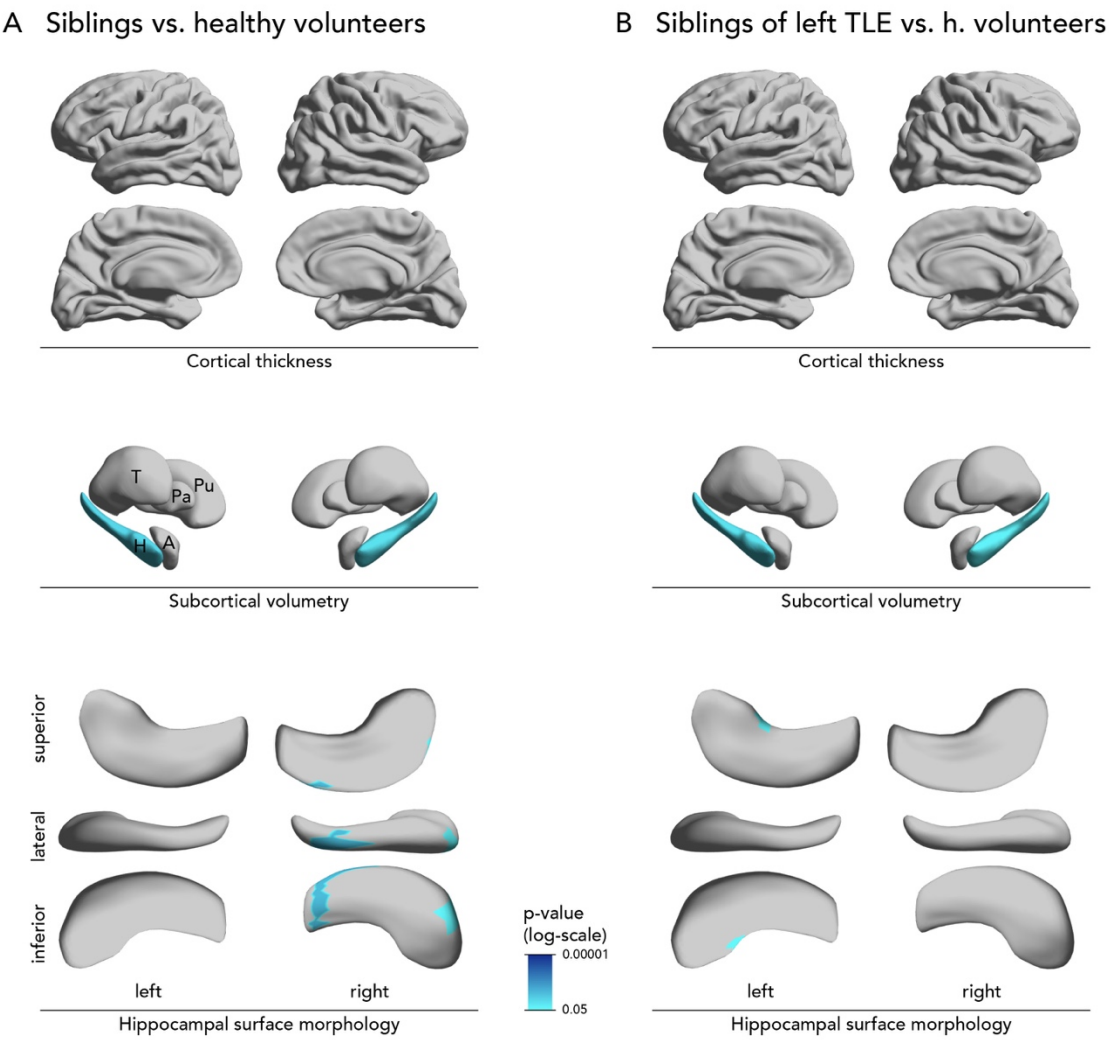
Shared hippocampal abnormalities in sporadic temporal lobe epilepsy patients and their siblings.

-- ONLINE SUPPLEMENT --

Contents:

1. Sensitivity analysis excluding one sibling with abnormal EEG

We conducted a sensitivity analysis excluding one sibling who had an abnormal EEG. Excluding this dataset did not alter the overall results - see figure below.



2. Clinical factors and brain morphology in patients with TLE

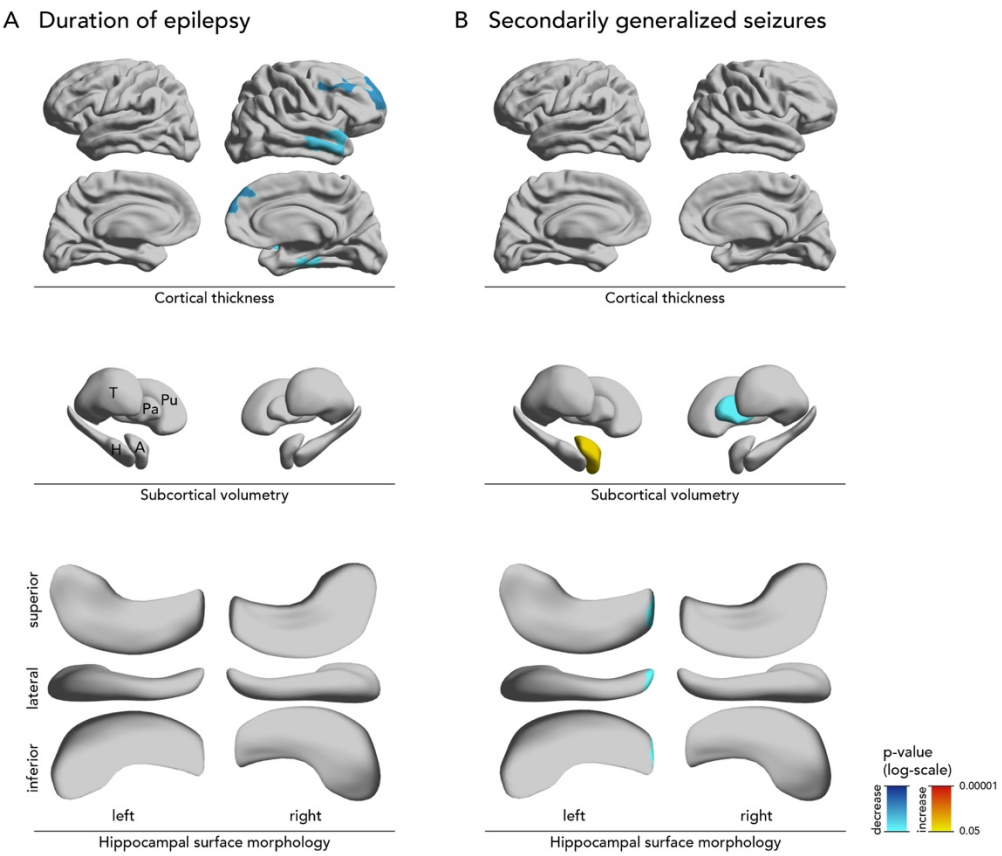
In the overall group of all patients with TLE, we correlated cortical thickness, subcortical volumetry, and hippocampal surface morphology with duration of epilepsy, history of secondarily generalized seizures, seizure frequency, and number of antiepileptic drugs (AEDs). Analyses were performed in accordance with the methods outlined in the original manuscript and all findings were corrected for age, sex, level of education, and MMSE scores and p-values were reported after correction for multiple comparisons using random field theory.

We found a significant association of cortical thinning with longer duration of epilepsy (corrected for age) in the right superior and middle frontal gyri (883 vertices, 8.1 resels, p=0.0008), right middle and superior temporal gyri (482 vertices, 5.0 resels, p=0.005), and the right parahippocampal gyrus (286 vertices, 3.7 resels, p=0.02). Duration of epilepsy did not correlate with volumes of subcortical structures or the surface morphology of the hippocampus.
These results support the concept that there is a genetic contribution to hippocampal sclerosis and that hippocampal abnormalities occurs early during the disease course, whereas cortical thinning is typically acquired (see also Alhusaini et al., Neurology, 2019) and worsens with longer duration of epilepsy (Whelan et al. Brain 2018, Galovic et al. JAMA Neurol 2019).

Patients with a history of secondarily generalized seizures (SGS) had a smaller volume of the right pallidum (history of SGS median 1.83 ml, IQR 1.78-1.92, vs. no SGS 1.98 ml, IQR 1.83-2.23, ß=-0.15 [95% CI -0.60 to 0.18], p=0.02, p_{BS}=0.15) and focal atrophy in the left hippocampal tail (34 points, 1.7 resels, p=0.046). This observation is in accordance with the role of subcortical structures in generating generalised seizures (Bertram et al. Epilepsia 2001, Blumenfeld et al. Brain 2009, Gale Journal of Clinical Neurophysiology 1992, Norden et al. Epilepsy & Behaviour 2002). Additionally, we found increased volume of the left amygdala in patients with history of SGS (history of SGS median 2.02 ml, IQR 1.77-2.08, vs. no SGS 1.83 ml, IQR 1.65-2.07, ß=0.19 [95% CI -1.41 to 0.82], p=0.02, pBS=0.15). Similarly, increased volume of the amygdala was previously found in cases with SUDEP and high risk cases with frequent generalized seizures (Wandschneider et al. Brain 2015, Allen et al. Epilepsia 2019). However, it is important to note that the effects in the right pallidum and in the left amygdala were non-significant after bootstrapping, suggesting that the observed effects might not be robust in different sample compositions and might not be generalizable.

There was no association of cortical or subcortical changes with seizure frequency or number of antiepileptic drugs. It has been suggested that neurodegeneration in epilepsy could represent an intrinsic pathological process underlying epilepsy that is not dependent on seizures (Galovic et al. JAMA Neurol 2019, Alvim et al. Epilepsia, 2016).

See figure below for detailed results.


A Duration of epilepsy
B Secondarily generalized seizures
Cortical thickness
Cortical thickness
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Subcortical volumetry
Subcortical volumetry
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p-value
(log-scale)
decrease
increase
0.00001
0.05
C Seizure frequency
D Number of antiepileptic drugs
Cortical thickness
Cortical thickness
Subcortical volumetry
Subcortical volumetry
Hippocampal surface morphology
Hippocampal surface morphology

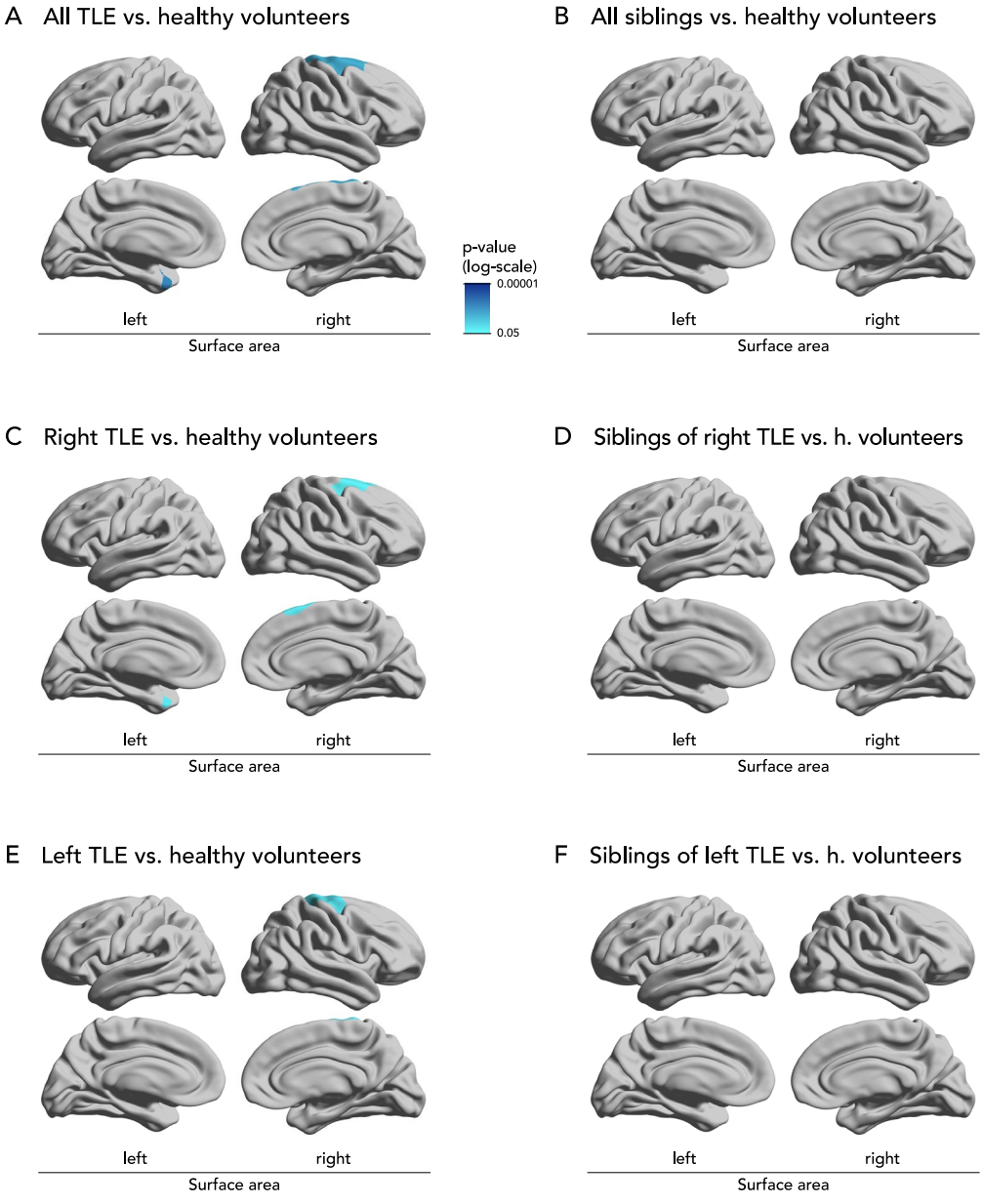
3. Surface area abnormalities in patients with TLE and siblings

We extracted surface area according to previous studies using the *recon-all* command implemented in FreeSurfer (version 6.0.0; http://surfer.nmr.mgh.harvard.edu). Surface area maps were transformed into template space and smoothed with a 15mm FWHM kernel. The remaining statistical processing was performed as outlined in our manuscript.

Patients with TLE showed abnormal cortical surface area in the left entorhinal cortex (378 vertices, 4.7 resels, p=0.0004) and the right superior frontal gyrus (5384 vertices, 3.7 resels, p=0.002). Patients with right TLE showed abnormal cortical surface area in the left entorhinal cortex (268 vertices, 2.5 resels, p=0.02) and the right superior frontal gyrus (3103 vertices, 2.3 resels, p=0.03). Patients with left TLE showed abnormal cortical surface area in the right superior frontal gyrus (3428 vertices, 2.5 resels, p=0.02).

The involvement of superior frontal and anterior temporal areas is similar to that described in Alhusaini et al. Cereb Cortex 2016.

Conversely, we did not detect any areas of abnormal cortical surface morphology in siblings (all siblings or siblings of patients with left or right TLE) at our current statistical thresholds. See figure below.



4. Hippocampal volume in patients with TLE without hippocampal sclerosis on visual analysis

Visual analysis of hippocampal sclerosis (HS) can underestimate the presence of hippocampal atrophy. To address this point, we compared hippocampal volumes in patients with TLE without HS (6 with RTLE, 5 with LTLE) with healthy volunteers. Patients with RTLE without HS had significantly smaller right hippocampi compared to healthy volunteers (RTLE without visual HS 2.41 ml, IQR 2.05-2.74, vs. controls 2.86 ml, IQR 2.58-3.17, pBS= 0.04). Patients with LTLE without HS had significantly smaller left hippocampi compared to healthy volunteers (LTLE without visual HS 2.07 ml, IQR 2.01-2.38, vs. controls 2.71 ml, IQR 2.37-2.89, pBS= 0.04).

Previous studies considered temporal lobe epilepsy (TLE) with hippocampal atrophy (TLE-HA) and TLE with normal hippocampal volumes (TLE-NV) as part of a pathologic continuum (Coan et al., 2009; Bernhardt et al., 2010; Bernhardt et al., 2013), and our results further support this concept. Therefore, the inclusion of both patients and siblings with TLE-HA and TLE-NV permits the generalizability of our findings to all TLE cases.

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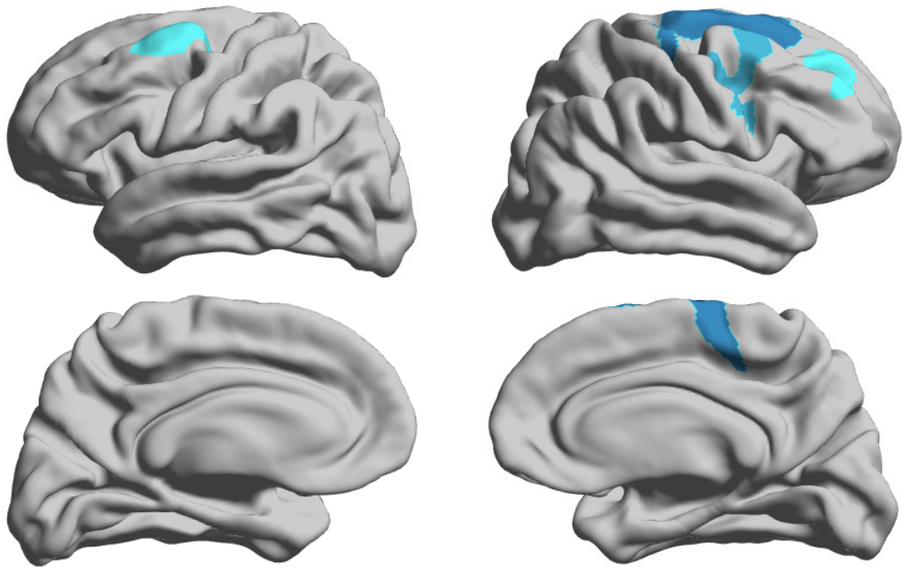
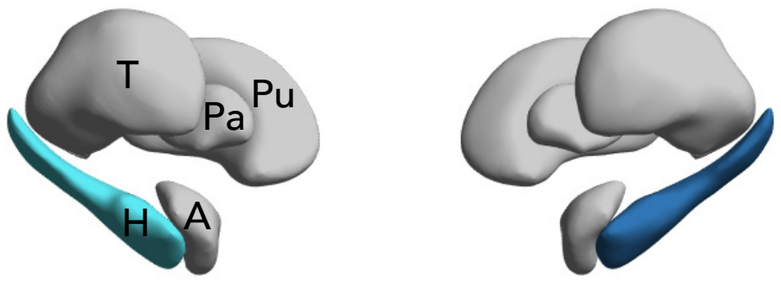
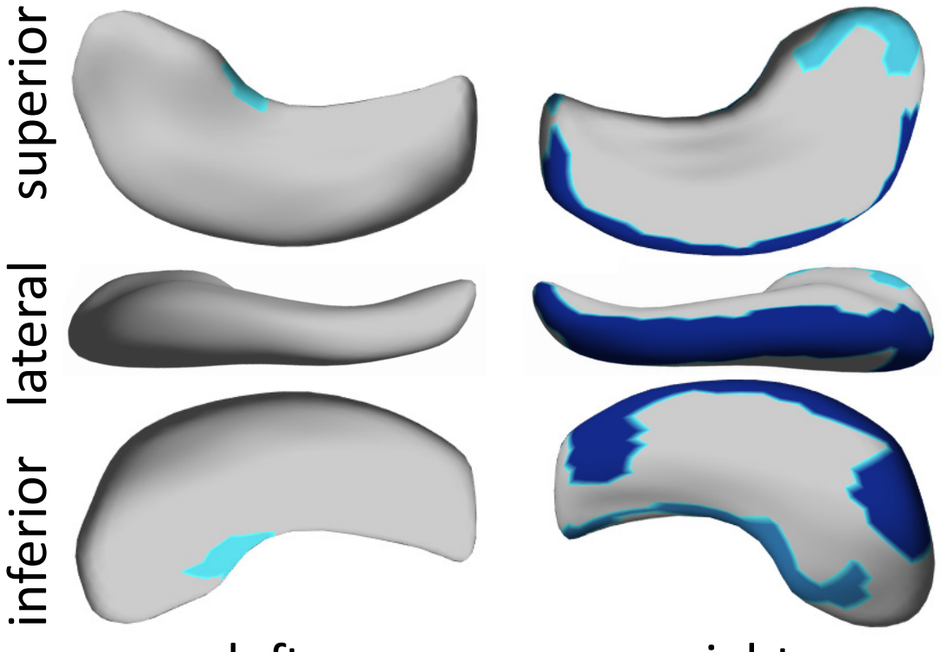
A TLE vs. healthy volunteers



Cortical thickness

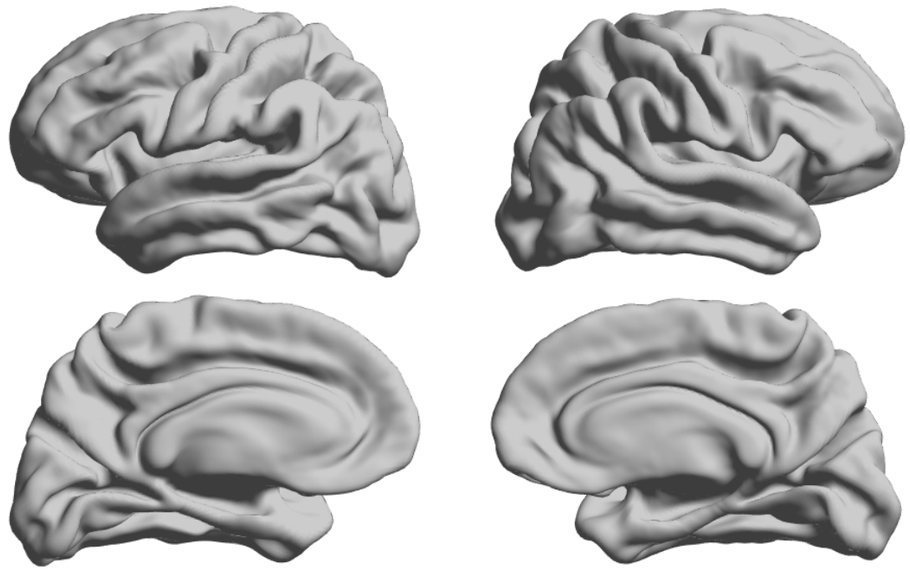


Subcortical volumetry



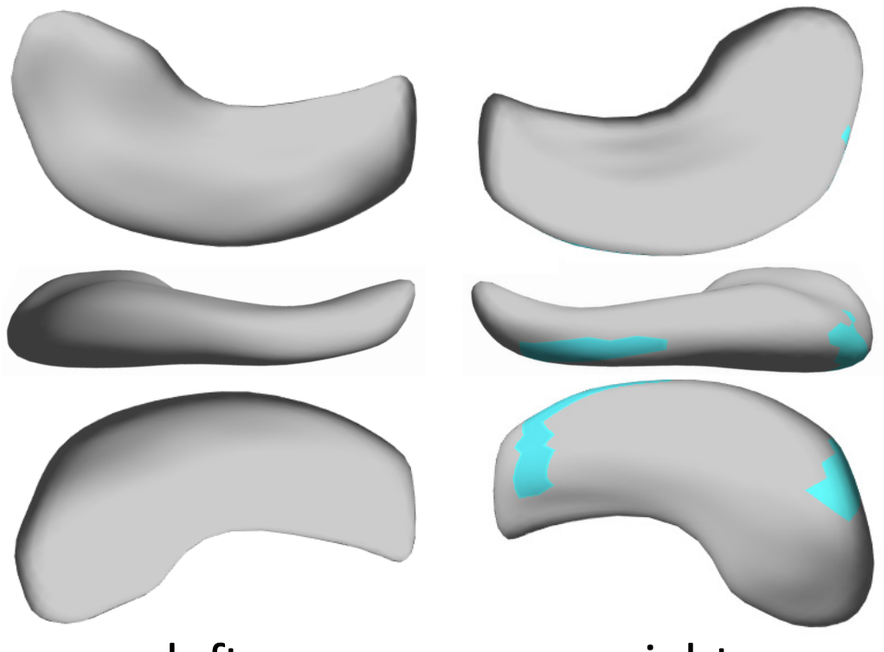
Hippocampal surface morphology

B Siblings vs. healthy volunteers

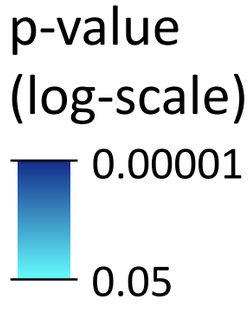


Cortical thickness

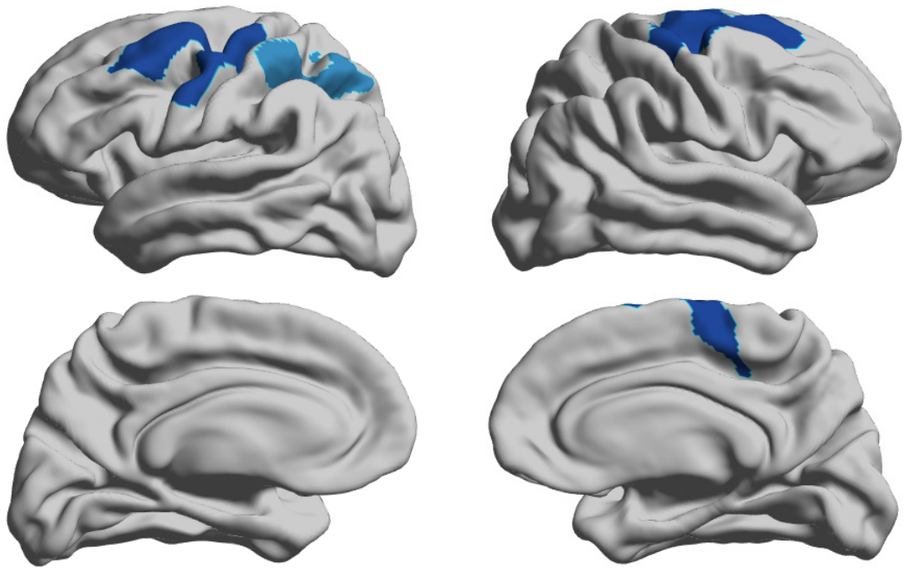
Subcortical volumetry



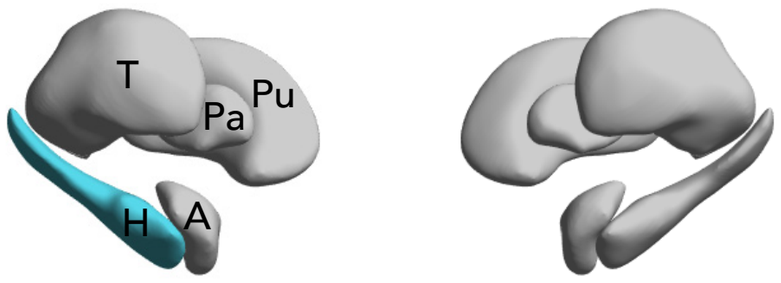
Hippocampal surface morphology



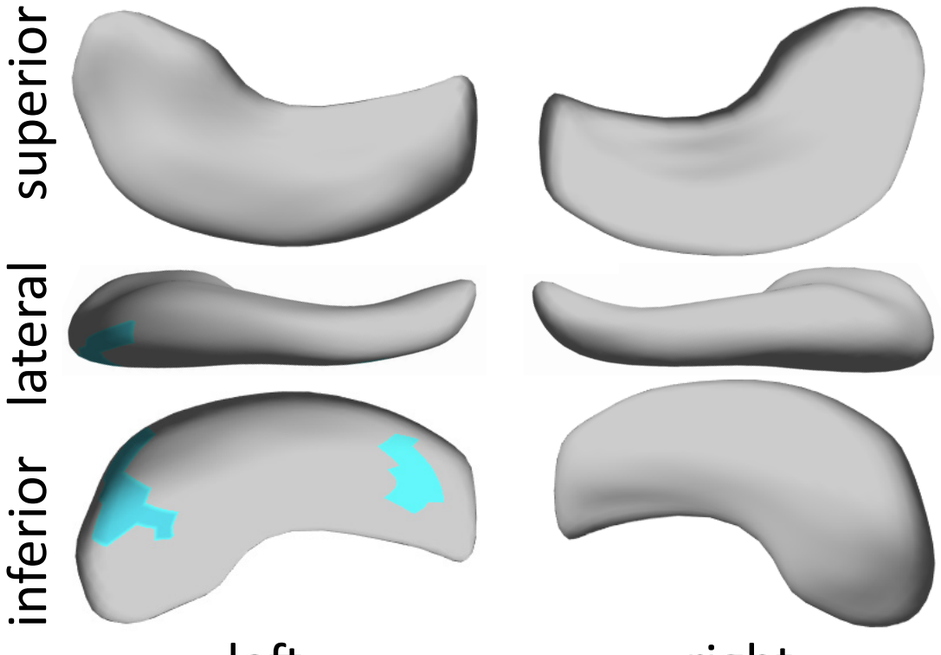
A Left TLE vs. healthy volunteers



Cortical thickness

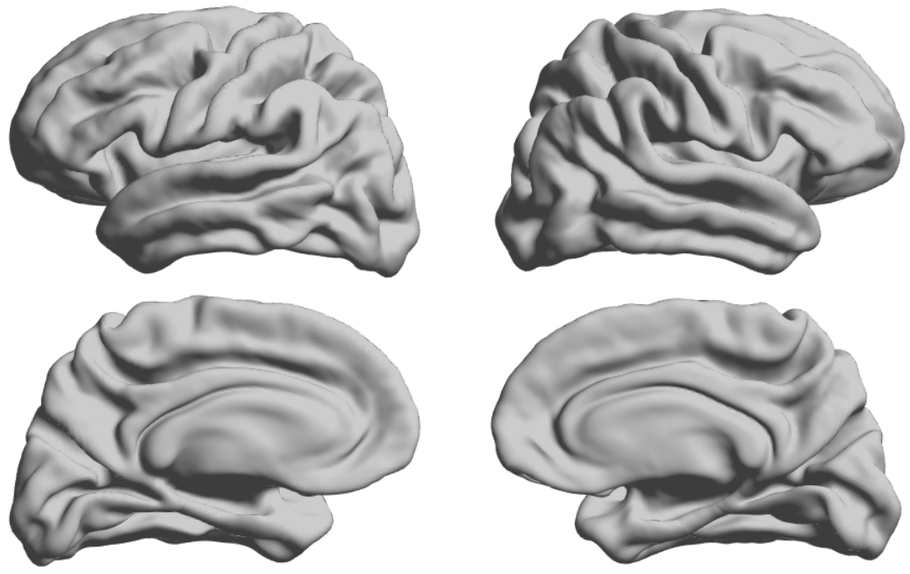


Subcortical volumetry



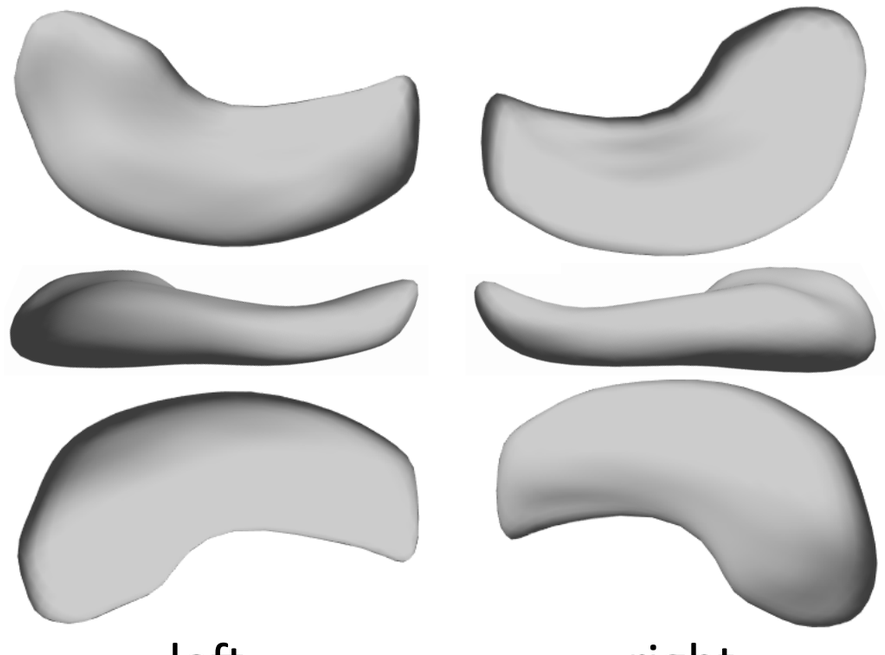
Hippocampal surface morphology

B Siblings of left TLE vs. h. volunteers

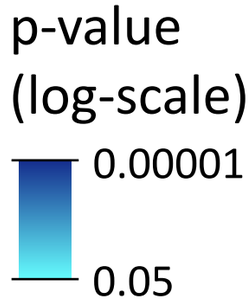


Cortical thickness

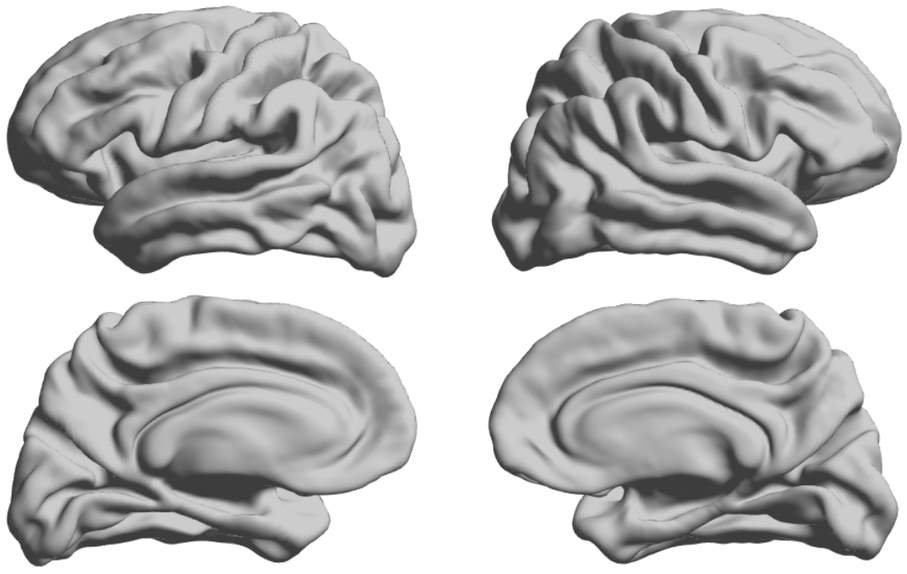
Subcortical volumetry



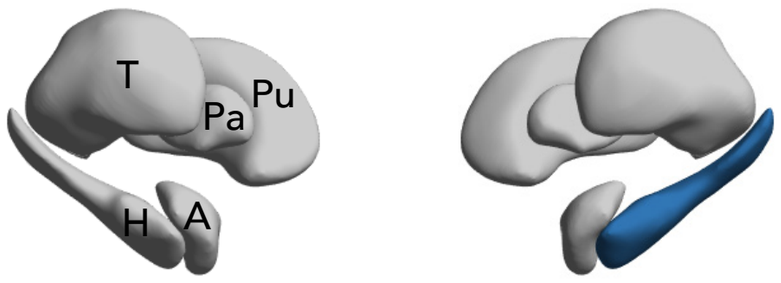
Hippocampal surface morphology



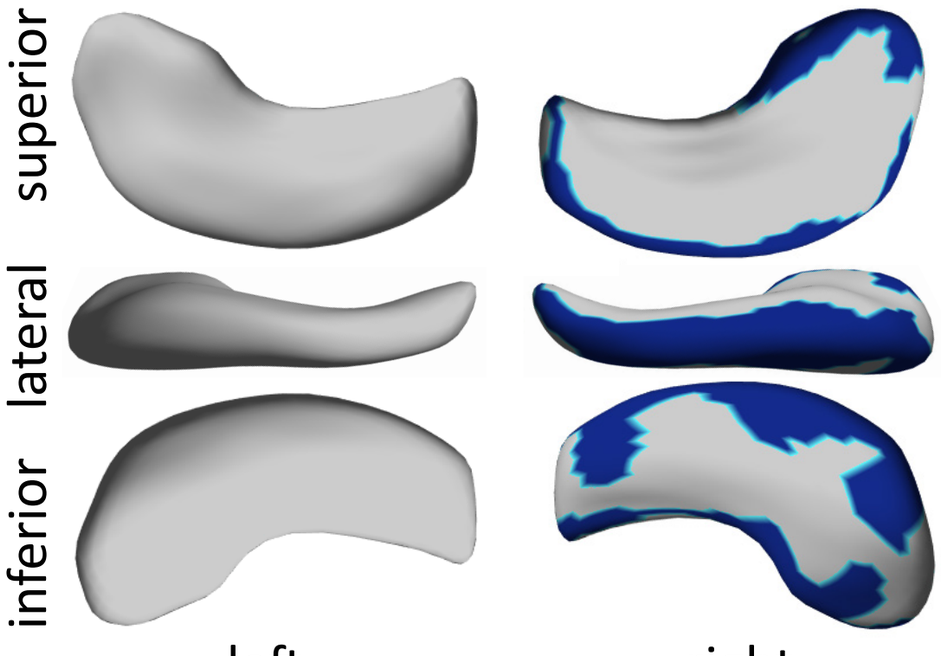
A Right TLE vs. healthy volunteers



Cortical thickness

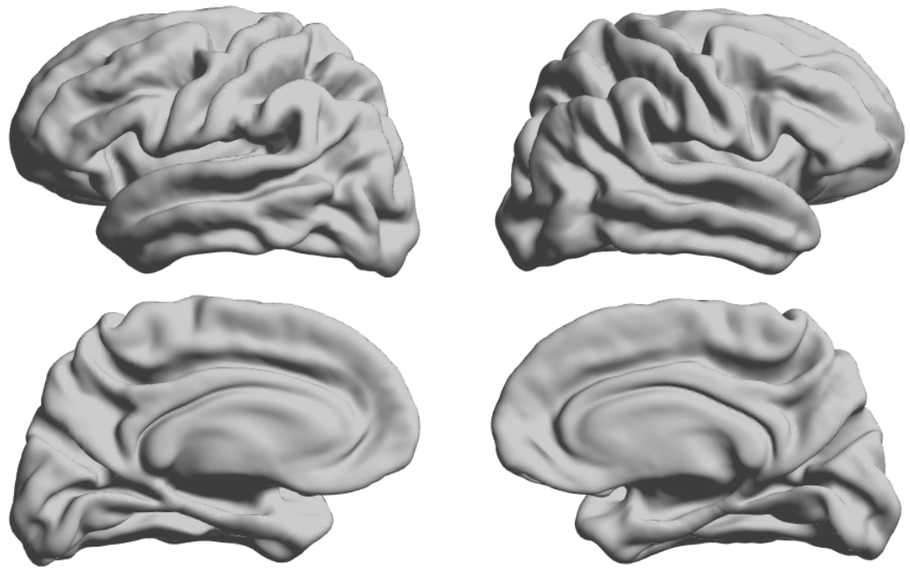


Subcortical volumetry



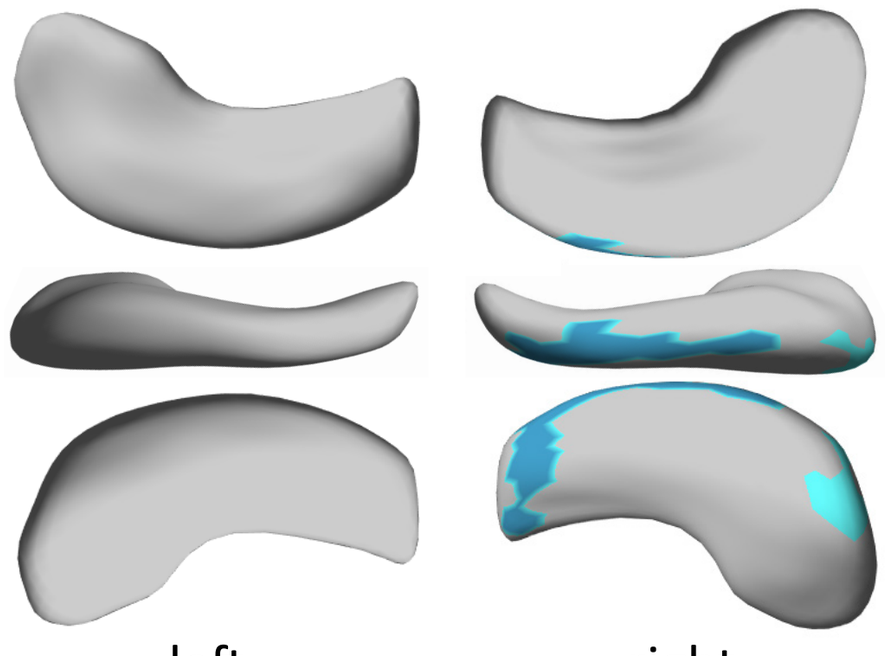
Hippocampal surface morphology

B Siblings of right TLE vs. h. volunteers



Cortical thickness

Subcortical volumetry



Hippocampal surface morphology